New ultra-high-throughput capability for biocatalyst screening

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Los Alamos researchers in the Bioscience Division and collaborators created a new capability for rapidly discovering—and evolutionarily enhancing—biocatalysts needed in the production of food, pharmaceuticals, specialty chemicals, renewable energy, and environmental cleanup.

Called Smart Microbial Cell Technology, the capability is based on a custom-made sensor–reporter gene circuit. When coupled with the high-throughput capability of flow cytometry, the technology can screen whole cell or enzyme biocatalysts at a rate of about one million per day.

“The limiting factor in biocatalyst screening has been the throughput, and our technology addresses that limitation. It is orders of magnitude faster than current state-of-the-art techniques, such as screening using micro-titer plates,” said Ramesh Jha, the technology’s technical lead.

Biocatalysts are specific, like a key in a lock

Biocatalysts do not fall into the one-size-fits-all category. Each reaction requires its own specific and optimized biocatalyst to help transform molecules into final products. But industrially useful reactions suffer from non-optimal activity and stability of biocatalysts, and nature has barely evolved anything for anthropogenic molecules, such as plastics or pesticides. There is a significant need for precise and optimized biocatalysts that can push each of these critical reactions.

With each reaction needing a specialized biocatalyst, pace becomes an issue. But it takes time to discover a biocatalyst that possesses gain-of-function mutations because these mutations are extremely rare and finding them is like searching for a needle in a haystack. It requires hunting through large libraries of possible biocatalysts and possible mutations for any given performance enhancement.

The Smart Microbial Cell Technology makes the screening process faster and simpler. For example, in a recent paper by the researchers, this technology was used to

enhance muconate production three-fold. Muconate is vital to the non-petrochemical production of polymer products, such as nylon and polyethylene terephthalate (PET). Their success was based on the custom development of a muconate sensor, which led to the discovery of tunable regions in the biocatalyst. From there, the researchers were able to build a more productive biocatalyst.

Other benefits of the new technology

The Smart Microbial Cell Technology is an ultrafast screening method for biocatalysts, and at the same time, it's extremely cost effective and simple. It requires only a fraction of the chemical reagents and consumables used in other methods. For example, what can be accomplished with a traditional method using 100,000 plastic 96-well micro-titer plates and 1,000 L of reagents can be accomplished with the new technology in a single small tube with 1 mL of reagents. It is a significant economic improvement.

The needed equipment and personnel are also more streamlined. Smart Microbial Cell Technology does not require complex machinery or highly trained personnel. It can be performed with a flow cytometer to boost the throughput, but it can also be performed without it, albeit with a slight drop in throughput. It's a flexible technology. This removes yet another bottleneck in the biocatalyst screening process while offering more accurate direct detection of the biocatalytic reaction.

"With an ultra-high-throughput capability, the Smart Microbial Cell Technology will pave the way for discovery and *de novo* synthesis of novel biocatalysts, which can be applied for biosynthesis and bioremediation of xenobiotic molecules with an effort to move towards a sustainable environment," said Jha.